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ANTENNULAR SENSILLA OF THE BRINE SHRIMP, *ARTEMIA SALINA*

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Cuticular sensilla of arthropods have been extensively studied in members of the class Insecta, and combined ultrastructural, physiological, and behavioral data have contributed greatly to our understanding of the functions and biological significance of such sensory structures (McIver, 1975; Slifer, 1970). By comparison, similar sensilla of members of the class Crustacea have received much less attention, and the majority of recent accounts have dealt solely with higher crustaceans, such as decapods (*e.g.*, Ball and Cowan, 1977; Snow, 1974; Tazaki, 1977; Vedel and Clarac, 1976). The present paper describes cuticular sensilla found on the antennules of the brine shrimp *Artemia salina,* a primitive crustacean belonging to the subclass Branchiopoda. Two distinct types of adult antennular sense organs are characterized by means of scanning and transmission electron microscopy. On the basis of the morphological findings, together with results from dye-penetration experiments, possible functions of the sensilla are discussed. Since the antennules of the naupliar larva of *Artemia* also bear setae that may be sensory in function, these larval structures were studied as well. Their external morphology is compared to that of the two types of sensilla found on antennules of adult brine shrimp.

Materials and Methods

Source of animals

Specimens of live adult *Artemia salina* (L.) were obtained from a commercial supplier (Living World, San Francisco Bay Brand, Newark, California) or from private cultures. To the best of our knowledge, all brine shrimp were from the San Francisco Bay area or had been cultured from eggs collected there. Live nauplii were hatched from brine shrimp eggs obtained commercially (Living World Hatch Mix) and were fixed no later than 30 hr after hatching.

Scanning electron microscopy (SEM)

A variety of fixation procedures was employed to preserve adult brine shrimp. The following three methods were most often used and yielded favorable specimens for SEM:

Method 1. Whole adult shrimp were fixed in 10% acrolein in 0.1 M sodium cacodylate for one hr at room temperature and then washed and stored in cold 0.1 M sodium cacodylate for periods ranging from three days to one month. Prior to osmication, the head of each animal was cut off with iridectomy scissors. The

heads were post-fixed in 2% osmium tetroxide in 0.1 M sodium cacodylate for 1.5 hr at room temperature and rinsed quickly six times in water. Further osmication of the tissue was done by means of a thiocarbohydrazide (TCH) technique modified from that of Malick, Wilson, and Stetson (1975). The specimens were subjected to the following sequential treatments: immersion in fresh, saturated, aqueous TCH for 30 min; six rinses in water; immersion in aqueous 2% osmium tetroxide for 2 to 3 hr; six rinses in water; immersion in TCH for 30 min; six rinses in water; immersion in 2% osmium tetroxide for 3.5 hr; six rinses in water. In some instances the above procedure was abbreviated by omitting the last four steps.

Method 2. Whole adults were fixed initially in 4% acrolein and 2% glutaraldehyde in 0.1 M sodium cacodylate for 5 hr at room temperature. The specimens were then transferred to 2% glutaraldehyde in the same buffer for storage in a refrigerator for two months. Prior to osmication, the animals were decapitated and the heads rinsed in buffer for at least 1 hr. The remainder of the processing procedure was similar to that described in the preceding paragraph.

Method 3. Whole adult shrimp were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate for 18 hr at room temperature and then refrigerated for five months in the same solution. Prior to osmication, the shrimp were decapitated and the heads rinsed in buffer overnight. The tissue was osmicated by the lengthy procedure described in the first method.

Nauplii were fixed in 2% osmium tetroxide in 0.1 M sodium cacodylate for 5 hr at room temperature. Next, the specimens were rinsed several times in buffer and stored overnight in cold buffer.

After osmication, all SEM specimens were dehydrated in ethanol and critical-point dried with carbon dioxide. Finally, the dried tissue was mounted on aluminum stubs with tape or silver conductive paint and coated with gold in a sputtering device (Technics, Inc.). Specimens were examined and photographed in a Hitachi HHS-2R scanning electron microscope.

Transmission electron microscopy (TEM)

Observations undertaken with TEM were limited in scope and served mainly to clarify the organization of the cuticular layer found at the base of the adult sensilla. Heads of adult brine shrimp were fixed for 15 to 16 hr in either of the following two solutions: 2% acrolein, 5% glutaraldehyde in 0.12 M sodium cacodylate, or 2% acrolein, 4% glutaraldehyde in 0.12 M sodium cacodylate. The tissue was then rinsed for 4.5 hr in 0.2 M sucrose in 0.12 M sodium cacodylate, post-fixed for 2 hr in 2% osmium tetroxide in 0.1 M sodium cacodylate, dehydrated in ethanol, and embedded in Epon epoxy resin. Ultra-thin sections were cut with a diamond knife, stained sequentially with aqueous uranyl acetate and lead citrate, and examined in an RCA EMU 2A or 2C electron microscope.

Dye-penetration experiments

The permeability of antennular sensilla was investigated by means of Slifer's (1960) crystal violet method, a technique that has been used in identifying chemo-

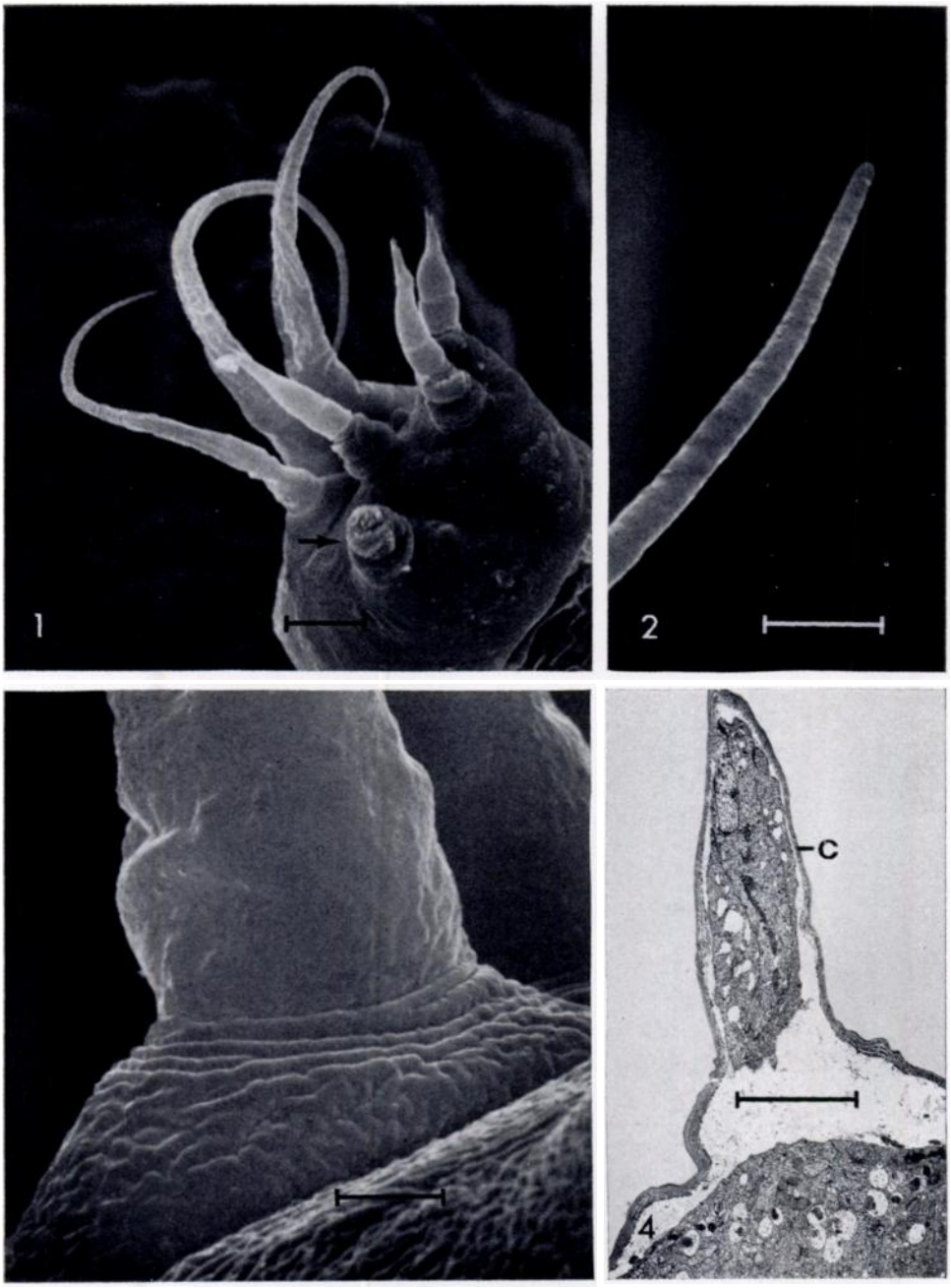

FIGURE 1. Scanning electron micrograph of tip of antennule of adult brine shrimp, showing two types of sensilla. Note three long, curved sensilla (type 1) and three shorter sensilla (type 2). The shaft of a fourth type 2 sensillum is missing, and only the basal knob-like elevation remains (arrow). Bar is 10 μm.

receptors in insects (Slifer, 1970). Whole adult shrimp were fixed in 3.7% formaldehyde in 0.1 M sodium cacodylate for 30 hr, washed overnight in water, and immersed in 0.5% crystal violet for periods of time varying from 1 min to 14 hr. Each animal was rinsed quickly in two or three changes of water to remove excess dye, and then the antennules were examined immediately by light microscopy. Nauplii were studied in the same way, except that fixation was carried out in a 3.7% formaldehyde solution made with hatch water.

RESULTS

Electron microscopy: adult brine shrimp

The tip of each antennule of an adult *Artemia* possessed a terminal cluster of sensory setae (Fig. 1). Two morphologically distinct kinds of sensilla, here designated type 1 and type 2, were present within a cluster. Type 1 sensilla were longer (43 to 80 μm in length) and, as seen in scanning electron micrographs, simpler in external morphology (Figs. 1–3). They were greatest in diameter at the base, where they originated from the tip of the antennule. Here, where the cuticle of the sensillum became continuous with the cuticle of the antennule proper, there appeared to be no socket or any other kind of articular specialization, although the cuticle of the sensillum and antennule did differ in surface rugosity. Transmission electron micrographs of ultra-thin sections also failed to reveal the presence of any articular modification of the cuticle at the base of the type 1 sensillum (Fig. 4). When the same kind of sensillum was examined more distally by SEM, the shaft of the sensory seta was seen to decrease very gradually in diameter and terminate as a finely tapered tip, which lacked pores or other salient morphological features capable of being resolved by scanning electron microscopy.

Type 2 sensilla (Figs. 1, 5–7) differed from the type 1 sensory setae in a number of respects. Type 2 sensilla were much shorter; the shaft length, as seen in scanning electron micrographs, was only 12 to 23 μm. The base of the type 2 sensillum possessed a distinctive modification of the cuticle in the region of the origin of the shaft. By SEM the shaft appeared to originate from the center of a knob-like bulge of the antennule proper. Transmission electron micrographs of sectioned material revealed that the shaft was in fact recessed within the knob-like bulge (Fig. 7) and that inside the knob the cuticle of the shaft became continuous with a circular invagination of antennular cuticle. Moreover, the cuticle inside the cup-like invagination was much thicker than that of the nearby antennular surface (Fig. 7).

FIGURE 2. High magnification micrograph of distal end of type 1 sensillum of adult. Compare to Fig. 6. Bar is 2 μm.

FIGURE 3. Scanning electron micrograph of basal region of type 1 sensillum of adult. Compare to Fig. 4. Bar is 2 μm.

FIGURE 4. Transmission electron micrograph of oblique section through basal region of type 1 sensillum of adult. Note lack of any articular specialization where cuticle (c) of sensillum becomes continuous with cuticle of antennule proper. Bar is 5 μm.

The shaft of a type 2 sensillum differed from that of a type 1 sensory seta in possessing a shallow, annular furrow (Fig. 5) and a pore on the distal end (Fig. 6). The pore rim (average pore diameter, 0.4 μm) was highly variable in morphology from one sensillum to another. The edge of each pore displayed distinctive finger-like or leaf-like cuticular projections that varied in number, length, shape, and arrangement. No two sensilla appeared to be identical in this respect.

Every *Artemia* antennule examined in detail by SEM was found to possess three of the type 1 sensilla; however, the number of type 2 sensilla per terminal cluster was variable. Usually four or five of the latter were present, but one of

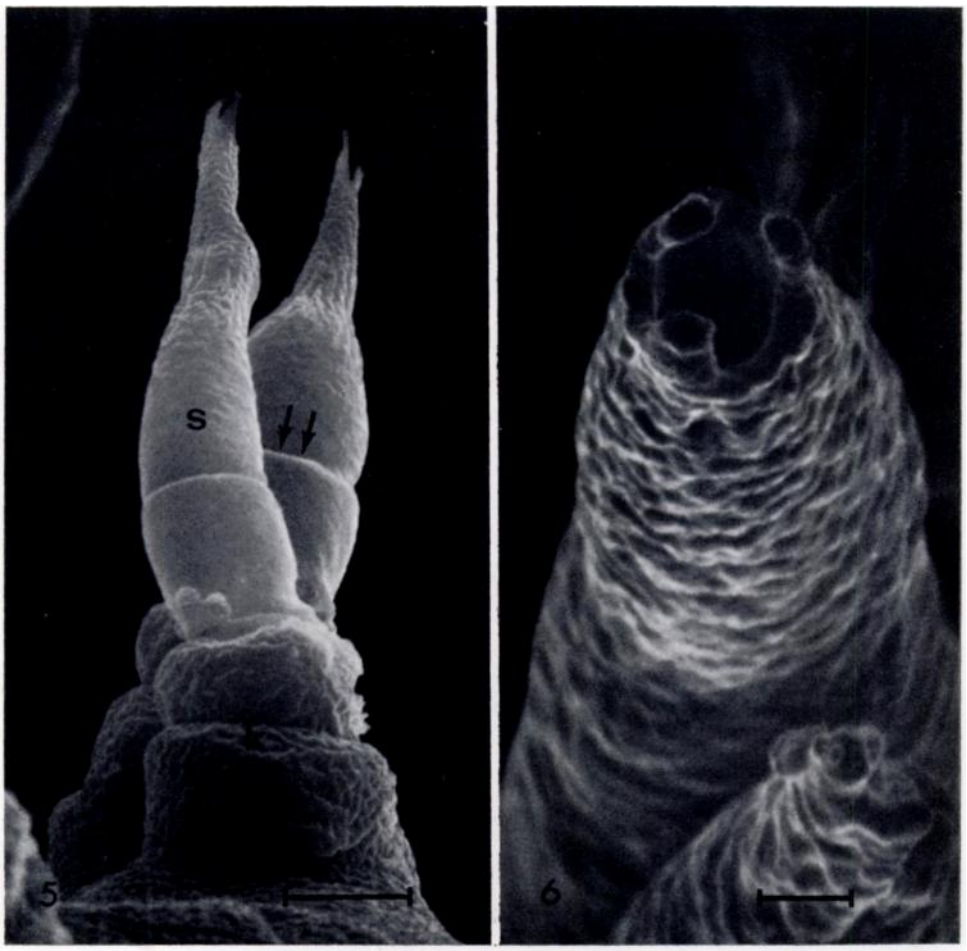

FIGURE 5. Scanning electron micrograph of type 2 sensilla of adult. Note the basal knob-like elevation (k) from which arises the shaft (s) of the sensillum. Arrows point to shallow, annular indentation of cuticle of shaft. Bar is 4 μm.

FIGURE 6. High magnification micrograph of distal end of type 2 sensillum. Note single pore on tip, and compare to Figure 2. Bar is 0.5 μm.

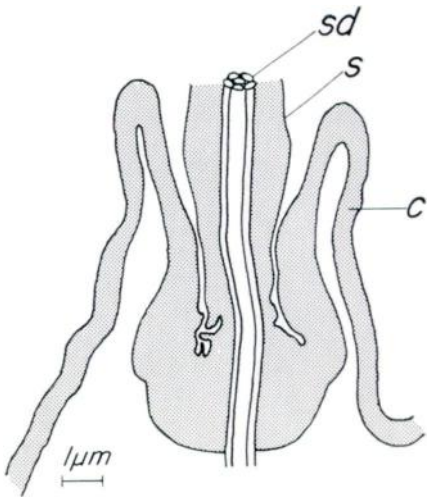

FIGURE 7. Diagram of base of type 2 sensillum as seen in thin section. Note the invagination of cuticle (c) within the basal knob-like elevation, as well as the recessed position of the proximal end of the shaft (s). Sensory dendrites (sd) are situated in lumen of shaft.

the antennules studied had only three. The number of type 2 sensilla per antennular cluster did not appear to be related to the sex of the individual, since totals of four and five were observed on antennules of both males and females.

Both types of sensilla were susceptible to damage or breakage during the protracted processing procedures used to prepare SEM specimens. However, even when a sensillum had broken off near the base of the shaft, it was relatively easy to recognize the site and type of the sensillum by the nature of the stump that remained. The numbers of sensilla reported in the preceding paragraph refer not to the number that remained intact after SEM processing but rather to the number of intact sensilla plus the number of damaged ones. Nothing is known about the percentage of damaged or missing sensilla normally present in the living state, but light microscopic observations of formalin-fixed animals indicated that much of the specimen damage observed by SEM occurred after the primary fixation step.

Electron microscopy: nauplii

The tip of each antennule of an *Artemia* nauplius normally bears three setae (Figs. 8–11). These larval setae varied in length from 13.5 μm to 66 μm and shared a number of features with the type 1 sensilla of adults. These features were: their number and general shape; the apparent absence of an articular modification of the cuticle at the base of the seta; and the absence of a pore at the distal end. Unlike type 1 sensilla of adults, however, the larval setae possessed short spines that were sparsely distributed over the general surface of the seta (Fig. 10). Such spines were sometimes situated quite close to the distal end of the seta.

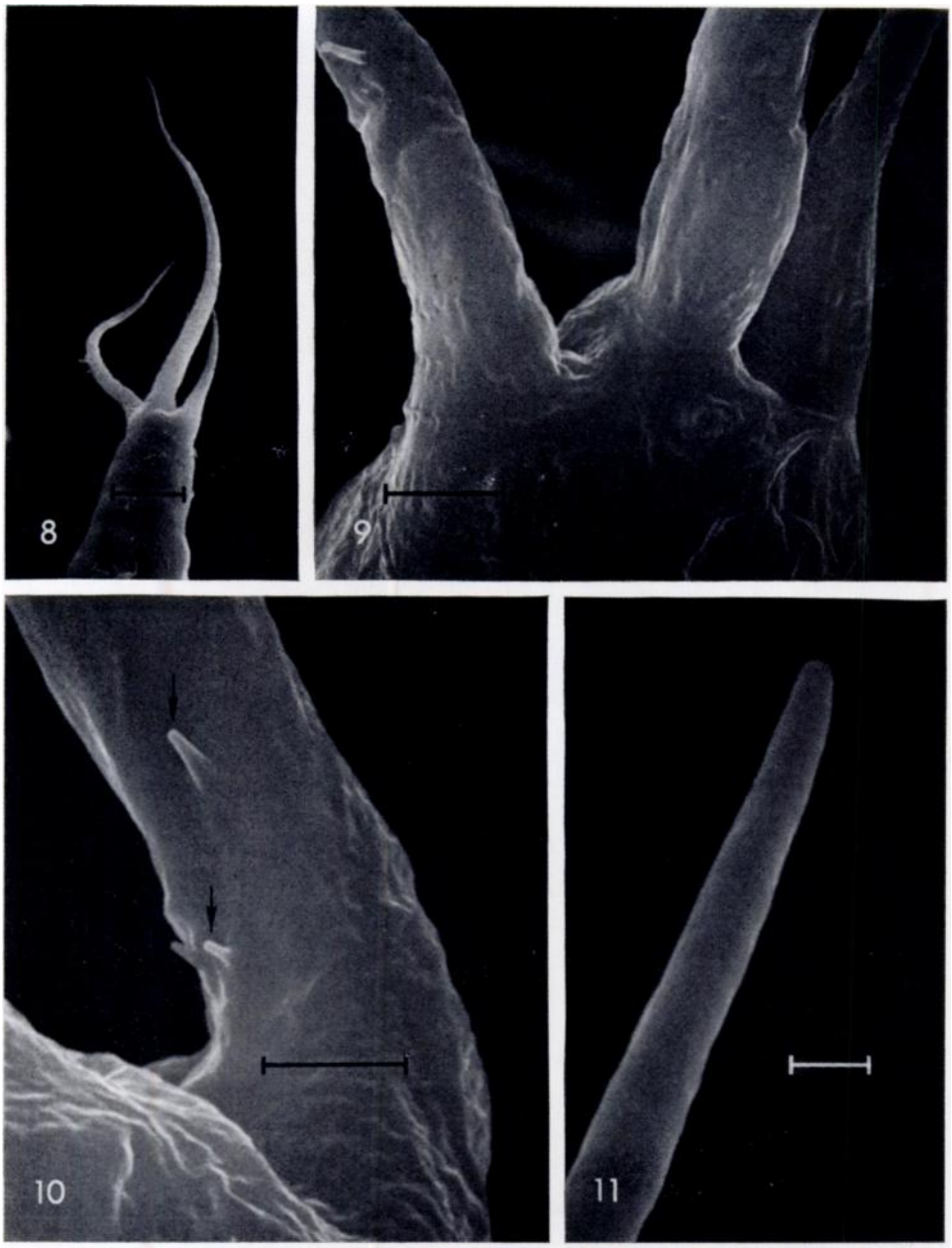

FIGURE 8. Scanning electron micrograph of tip of naupliar antennule (a). Note number and shape of antennular setae. Bar is 10 μm.

FIGURE 9. Tip of naupliar antennule showing basal region of naupliar setae. Bar is 3 μm.

FIGURE 10. Basal region of naupliar seta. Note short spines (arrows). Bar is 2 μm.

FIGURE 11. High magnification micrograph of tip of naupliar seta. Compare to Fig. 2. Bar is 0.5 μm.

Dye-penetration experiments

Light microscopic examination of antennules of adults immersed in aqueous crystal violet indicated that type 2 sensilla were permeable to the dye, whereas type 1 sensilla were not. At the shortest immersion time studied, *i.e.*, 1 min, the type 2 sensilla were stained, but even after immersion for as long as 14 hr, there was no evidence that crystal violet penetrated the type 1 setae.

With regard to naupliar larvae, there was no indication that the dye was able to enter the cuticle of the larval antennular setae. In some instances staining of the setae was seen, but all the observations made indicated that this staining occurred not by local penetration of the dye but rather by penetration elsewhere on the body surface, followed by internal diffusion of the dye to the antennules. Therefore, the larval setae resembled type 1 sensilla of adults in that both were impermeable to crystal violet solution.

Discussion

During the past decade, scanning electron microscopy has been used to study the external morphology of cuticular sensilla of members of the following major groups of crustaceans: decapods (Ball and Cowan, 1977; Mauchline, Aizawa, Ishimaru, Nishida, and Marumo, 1977; Shelton and Laverack, 1970; Snow, 1974; Tazaki, 1977; Tazaki and Ohnishi, 1974; Thomas, 1971; Vedel and Clarac, 1976), amphipods (Dahl, 1973a, b; Dahl, Emanuelsson, and von Mecklenburg, 1970), isopods (Nielsen and Strömberg, 1973), mysids (Guse, 1978), barnacles (Foster and Nott, 1969; Munn, Klepal, and Barnes, 1974), copepods (Fleminger, 1973; Strickler, 1975; Strickler and Bal, 1973), ostracods (Danielopol, 1971), and cladocerans (Dahm, 1976). These SEM studies have increased our knowledge of the morphological diversity that exists among crustacean sensilla, but for most of the sensilla examined there are no conclusive physiological data concerning function. The same is true for the antennular setae of brine shrimp. The following discussion of the possible functions of these structures is speculative, and it should be remembered that the specific functions of the antennular sensilla of *Artemia* are not known and at the present time can not be deduced with any certainty from morphological features alone.

In the present study, a sensory function has been assumed for type 1 and type 2 antennular setae of adults because both kinds are known to be innervated (Tyson, unpublished observations). Evidence that the antennules of adult brine shrimp are mechanosensitive comes from a study by Lent (1977), which deals with the coordination of metachronal limb movements in male-female pairs. Lent found that pulsatile water movements directed to the head of a brine shrimp could elicit a change in the metachronal frequency of limb movements of that individual and that pulse responsiveness was lost when both antennules were removed. It is feasible that either type 1 or type 2 sensilla are the mechanoreceptors that Lent suggested are involved in coordinating movements during tandem swimming. In mechanoreception the primary step in the transduction process is generally thought to involve mechanical deformation of a sensory cell process (Thurm, 1965). Deflection or bending of either type 1 or type 2 sensilla could conceivably result in the appropriate stimulation of sensory dendrites. In the case of type 2

sensilla, the presence of a basal articular modification may be significant, since it is possible that this structural specialization provides some flexibility to the base of the shaft. Movement at the site of articulation might result in deformation of the sensory dendrites that enter the shaft in this region. It is also plausible, however, that some flexibility exists at the base of the sensillum without producing concomitant mechanical stimulation of sensory processes. In this regard, it should be pointed out that socket-like modifications have been observed at the base of certain crustacean aesthetascs, and these sensilla are thought to be chemosensory in function (Snow, 1973, 1974).

The type 2 sensilla of adult *Artemia* may in fact be chemoreceptors. Both the presence of a terminal pore and the demonstrated permeability to crystal violet support such a hypothesis. In the case of insect cuticular sensilla, the presence of these two features is considered to be strongly diagnostic of a chemosensory function (Slifer, 1970). In the case of crustaceans, however, it is not clear how indicative of chemoreception the presence of a pore is, because pores have been observed in setae suspected to be mechanosensory, rather than chemosensory (Snow, 1974). In the preceding discussion the possibility has been explored that type 2 sensilla may be either mechanoreceptors or chemoreceptors. It is also feasible that type 2 sensilla are dual in function and each serves in both mechanoreception and chemoreception. In crustaceans the presence of sensilla with a dual function has not been documented, but such sensilla are known to occur in insects (McIver, 1975).

With respect to the nauplii of *Artemia,* it has long been recognized that naupliar antennules bear setae (Anderson, 1967; Joly, 1840), but it is not known whether or not these larval structures are innervated. Nor is anything known about how they are related, in a developmental sense, to the antennular setae of adults. In the present study, naupliar antennules were examined by SEM in order to permit a comparison of the larval and adult structures. The naupliar setae bore no resemblance to the type 2 sensilla of adults, but they were similar to type 1 sensilla in overall shape, in lacking a terminal pore, and in being impermeable to crystal violet. Another salient feature common to both the naupliar setae and the adult type 1 sensilla was their number per antennule. A total of three setae was normally present on each naupliar antennule, and every adult antennule examined by SEM likewise possessed three type 1 sensilla. The type 2 sensilla of adults were, on the other hand, more variable in number, and typically there were four or five present per antennule. It is possible that the three naupliar setae represent a developmental stage in the formation of three of the adult sensilla. If this be true, all available observations support the hypothesis that the larval structures are developmentally related to type 1 sensilla, rather than to type 2.

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Summary

1. Scanning electron microscopy was used to characterize the external morphology of setae found on the antennules of adults and nauplii of the brine shrimp, *Artemia salina* (L.). The permeability of the antennular setae was studied by means of Slifer's crystal violet method.

2. Each antennule of an adult brine shrimp possessed a terminal cluster of sensory setae. Within a cluster there were two morphologically distinct kinds of sensilla, here designated type 1 and type 2. Three type 1 sensilla were observed on every antennule examined. The number of type 2 sensilla per antennule was usually four or five.

3. Type 1 sensilla of adults were 43 to 80 μm long and simple in external morphology. They were widest at the base, decreased in diameter gradually, and terminated as a finely tapered tip. No pores were resolved by scanning electron microscopy.

4. Type 2 sensilla of adults were shorter (shaft length, 12 to 23 μm) and displayed a single pore at the tip (average pore diameter, 0.4 μm). In thin section they were seen to possess a distinctive articular specialization of the cuticle at the base of the seta.

5. Dye penetration experiments indicated that type 2 sensilla were permeable to aqueous crystal violet, whereas type 1 sensilla were not.

6. The antennular setae of nauplii resembled type 1 sensilla in general shape, in being impermeable to crystal violet, and in lacking a terminal pore and basal articular specialization. Moreover, a total of three setae was normally present on each naupliar antennule, and the same number of type 1 sensilla was found on each adult antennule examined. If the three naupliar setae represent a developmental stage in the formation of three adult sensilla, available observations suggest that the larval setae are developmentally related to type 1, rather than to type 2 adult sensilla.

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